

AD-A197 443

REPORT DOCUMENTATION PAGE

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1a. REPORT SECURITY CLASSIFICATION Unclassified			1b. RESTRICTIVE MARKINGS		
2a. SECURITY CLASSIFICATION AUTHORITY			3. DISTRIBUTION / AVAILABILITY OF REPORT Approved for public release and sale. Distribution unlimited.		
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE					
4. PERFORMING ORGANIZATION REPORT NUMBER(S) ONR Technical Report No. 10			5. MONITORING ORGANIZATION REPORT NUMBER(S)		
6a. NAME OF PERFORMING ORGANIZATION University of Utah		6b. OFFICE SYMBOL (If applicable)		7a. NAME OF MONITORING ORGANIZATION	
6c. ADDRESS (City, State, and ZIP Code) Department of Chemistry Henry Eyring Building Salt Lake City, UT 84112				7b. ADDRESS (City, State, and ZIP Code)	
8a. NAME OF FUNDING / SPONSORING ORGANIZATION Office of Naval Research		8b. OFFICE SYMBOL (If applicable)		9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER N00014-85-K-0712	
8c. ADDRESS (City, State, and ZIP Code) Chemistry Program, Code 1113 800 N. Quincy Street Arlington, VA 22217		10. SOURCE OF FUNDING NUMBERS			
		PROGRAM ELEMENT NO.		PROJECT NO.	WORK UNIT ACCESSION NO.
11. TITLE (Include Security Classification) Temperature-Induced Changes in Reversed-Phase Chromatographic Surfaces: C8 and C9 Polymeric Ligands					
12. PERSONAL AUTHOR(S) J. W. Carr and J. M. Harris					
13a. TYPE OF REPORT Technical		13b. TIME COVERED FROM 9/87 TO 7/88		14. DATE OF REPORT (Year, Month, Day) July 15, 1988	
15. PAGE COUNT 24					
16. SUPPLEMENTARY NOTATION					
17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)		
FIELD	GROUP	SUB-GROUP			
			Fluorescence spectroscopy of liquid/solid interfaces, alkylated silica surfaces.		
19. ABSTRACT (Continue on reverse if necessary and identify by block number) Attached.					
20. DISTRIBUTION / AVAILABILITY OF ABSTRACT <input checked="" type="checkbox"/> UNCLASSIFIED / UNLIMITED <input type="checkbox"/> SAME AS RPT <input type="checkbox"/> DTIC USERS			21. ABSTRACT SECURITY CLASSIFICATION Unclassified		
22a. NAME OF RESPONSIBLE INDIVIDUAL			22b. TELEPHONE (Include Area Code)		22c. OFFICE SYMBOL

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OFFICE OF NAVAL RESEARCH

Contract N00014-85-K-0712

R&T Code 413a001---01
Replaces Old Task #056-123

Technical Report No. 10

Temperature-Induced Changes in Reversed-Phase
Chromatographic Surfaces: C8 and C9 Polymeric Ligands

Prepared for publication in J. Chromatography

by

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July 15, 1988

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TEMPERATURE-INDUCED CHANGES IN REVERSED-PHASE
CHROMATOGRAPHIC SURFACES: C8 AND C9 POLYMERIC LIGANDS

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Summary

The temperature-dependent polarity of polymeric C8 and C9 stationary phases, preconditioned with acetonitrile and then contacted with water, is studied using the fluorescence of sorbed pyrene as a probe. The fluorescence vibronic band ratio, sensitive to the polarity of the probe surroundings, shows a temperature-dependent hysteresis which is indistinguishable from the retention behavior of these materials. The stationary phase polarity decreases when passing through the hysteresis, which is consistent with the loss of conditioning solvent from the interface but inconsistent with an extended structure for the alkyl ligands. Solvent retention in the stationary phase and its temperature dependence appears to require local ordering of the polymer phase.

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INTRODUCTION

One of the more challenging aspects of developing a comprehensive model for reversed-phase liquid chromatography has been to determine the role of the alkylated surface in the selective retention of solute molecules. While mobile phase effects dominate solute retention [1,2], the contribution of the surface to retention has been the subject of intensive investigation and interest [3-13]. These chromatographic experiments have provided insight into the structure and behavior of bonded hydrocarbon layers and have extended our ideas of the stationary phase beyond a homogeneous, hydrocarbon layer. A picture of the stationary phase as a dynamic surface, responding to conditions in the mobile phase and affecting solute retention, is beginning to emerge.

Models of the stationary phase as an alkyl "grass" or "bristles" [5] attached to the solid support, or as a hydrocarbon "blanket" covering the silica gel [3] are being replaced by models of a heterogeneous surface incompletely covered by chemically bonded alkyl ligands. Lochmüller and Wilder have suggested a microdroplet configuration for the bonded layer, where alkyl ligands are aggregated by hydrophobic interactions into clusters of organic material [4]. If the solute is sufficiently small to be contained within these clusters, the stationary phase would appear as islands of non-polar material distributed on a polar silica surface. The organization of bound ligands appears to influence shape selectivity of retention [11,12] and depends on the bonding to the silica surface, the degree of surface coverage, and the underlying substrate. Distribution of solvent components into the stationary phase has also been studied, and the resulting effects on solute retention have been described [6-10].

Transfer of organic modifier from the mobile phase into the stationary

phase is responsible for changes in the environment of a solute sorbed to the bound layer [13-16] and for changes in the phase volumes [6,9]. The intercalation of organic modifier into the stationary phase may, in addition to a change in the polarity of the bonded layer, be responsible for conformational changes in the surface as well. While studying the effects of temperature and conditioning solvent on polymeric C8, C9, and C10 stationary with a totally aqueous mobile phase, Gilpin and co-workers [7] noted an interesting hysteresis in the temperature dependence of solute retention, following preconditioning with organic solvent. They showed in a subsequent study that organic modifier, initially retained by the stationary phase when the mobile phase is abruptly changed from neat organic modifier to water, is released [8] when a sufficiently high temperature is reached. The temperature at which the solvent was released correlated with the alkyl chain length and functionality of the organic solvent [7,17]. A mechanism proposed to account for the observed behavior was that solvent molecules were trapped in the stationary phase by collapse of the alkyl ligands [7] into an aggregated, metastable state [17] when exposed to water. Raising the temperature of the stationary phase above a transition temperature was postulated to cause restructuring of the surface ligands into an extended or "bristle" configuration [7,8], releasing trapped organic modifier which is replaced water molecules between the hydrocarbon chains, now in an extended, more stable surface structure [17].

In the present work, the surface environments of C8 and C9 polymeric stationary phases, under conditions of the above temperature-dependent hysteresis, are examined using the fluorescence emission of sorbed pyrene as a probe. The vibronic band structure of the fluorescence from pyrene is quite sensitive to the polarity of the molecule's local environment [18]. As the

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solvent polarity around pyrene is increased, the intensity of the vibronic origin of the weak, highest energy L_b transition (Band I at 374 nm) is increased due to symmetry lowering perturbations from the solvent environment which allow mixing with the much stronger L_a transition dominating the third vibronic peak (Band III at 385 nm) [19]. As a result, the ratio of the intensities of the third and first peaks, III/I, varies inversely with increases in the polarity of microenvironment of the pyrene probe [14,18]. The vibronic band ratios of pyrene fluorescence have been used to study the interfacial microenvironments of polymeric and monomeric, C18 stationary phases by sorption of the probe to the hydrophobic surface [14-16]. The results of these studies showed the effects on C18 stationary phases of organic modifier and the heterogeneity of the sorption environments as a function of overlaying solvent. In the present work, the polarity of polymeric C8 and C9 stationary phases, preconditioned with acetonitrile and then contacted with water, is investigated as a function of temperature using sorbed pyrene as a probe. The results indicate that the polarity of the stationary phase decreases upon heating, which is not consistent with an extended structure for alkyl ligands in contact with the totally aqueous mobile phase.

EXPERIMENTAL

Chemicals. Pyrene was obtained from Aldrich and used after recrystallization from ethanol-water solution. Reversed-phase HPLC analysis of the compound using a 10 cm, 10 μ m particle column revealed no resolvable impurities. Acetonitrile (HPLC grade) was obtained from MCB and Burdick and Jackson. Water was purified in house using a Corning still (MP-1) and a Barnstead, four-cartridge Nanopure system. Decane and 1,2 ethanediol were obtained from Fisher and used without further purification. Polymeric C8 and C9 stationary phases were prepared in-house by reacting either n-octyltrichlorosilane or n-nonyltrichlorosilane with 10 μ m irregular silica (Partisil-10) having a surface area of approximately 320 m²g⁻¹ and a mean pore diameter of 9.6 nm before derivatization. The trichlorosilane reagents were obtained from Petrarch and used without further purification. The silica was stirred in water-saturated toluene for 2 hours. After filtering, dry toluene and the silane reagent were added to the silica and refluxed for 2 hours. Carbon loading of the resulting polymer phase was determined to be 8.4% and 7.5% for C8 and C9 respectively by M-H-W Laboratories (Phoenix, AZ).

Column preparation. The stationary phase was contained in a 1.5 mm ID precision bore quartz tube (Wilmad Glass Co.). The quartz microcolumn was supported in a brass cuvette assembly. Both the cell and the packing procedure have been described in detail in earlier publications [15,20].

Before an experiment, the stationary phase was conditioned by passing large volumes of acetonitrile through the column (approximately 50 mls or 2000 column volumes) at a flow rate of 1 ml/min. Pyrene was introduced to the column with a 50:50 (V:V) acetonitrile-water mobile phase. The concentration of pyrene in the mobile phase was adjusted to a value of ca. 3.0 μ M, which

gives a surface concentration low enough to avoid excimer formation (to assure that no probe-probe interaction are present) but great enough to emit a strong fluorescence signal with a small fraction of shot noise. When the pyrene concentration on the column reached equilibrium (as determined by a steady-state fluorescence signal), the mobile phase was changed to 100% water for the remainder of the experiment. The contributions to the fluorescence signal from pyrene in the mobile phase were extremely small due to the high capacity factor conditions for 100% water mobile phase conditions. Using in situ fluorescence measurement of the frontal elution of pyrene through a spectroscopic microcolumn, the capacity factor for pyrene on a C18 phase has been measured to be $k' = 6.0 \times 10^3$ [21]. While the lower carbon loading of the C8 and C9 stationary phases studied here would result in a factor of two or so smaller k' , the mass fraction of pyrene in the mobile phase under these conditions would still be less than 0.1%.

Fluorescence measurements. Emission spectra of pyrene sorbed onto the stationary phase were measured at 2.5 nm resolution on a Farrand model 201 spectrofluorimeter (Farrand Optical Co. New York, NY). The excitation wavelength used through the experiment was 335 nm. Fluorescence emission spectra were recorded on a strip chart. To obtain a measure of the stationary phase polarity, the intensity ratio of the third vibronic band to the highest energy, first band was calculated. The reported results were typically obtained from the average of 7 spectra. At a particular temperature, the precision of the vibronic band intensity ratios was normally 0.3% relative standard deviation.

The brass cuvette, when placed in the spectrometer, was in thermal contact with a circulating fluid bath (Haake, model L). The temperature of the cell

could be set between 10 °C and 110 °C (± 0.25 °C) using a Haake temperature controller (model D3). In a typical experiment, the column would be raised from 14 °C to 65 °C and then cooled down to approximately 15 °C. A series of spectra were recorded every 2.5 to 5.0 °C when the temperature was being increased and every 5.0 to 15.0 °C when the sample was cooling down. The magnitude of the temperature change between the series of spectra was determined by the behavior of the stationary phase; i.e. the rate of change of surface polarity. After a series of spectra at a particular temperature were recorded the system was allowed to reach the next temperature and kept there for 10 minutes to ensure satisfactory equilibration; during this period the solvent flow was discontinued. Aqueous mobile phase at a flow rate of 1.0 ml/min was then pumped through the column for 2 minutes to remove any released organic modifier. The flow rate was reduced to 0.5 ml/min while the spectra were being recorded.

The temperature dependence of pyrene vibronic bands was also measured in free solution in four different solvents in a standard 1 cm quartz fluorescence cell (Wilmad Glass Co.). The solvents used covered a wide range of polarity: decane, 1,2 ethanediol, acetonitrile, and water. The conditions were the same as on-column experiments except that the solution was not flowing. Spectra were obtained from 15 °C to 105 °C for decane and 1,2 ethanediol, between 15 °C and 75 °C for acetonitrile and between 15 °C and 95 °C for water.

RESULTS AND DISCUSSION

Temperature dependence of stationary phase polarity. The effect of temperature on the fluorescence vibronic band ratio of pyrene sorbed to a C8 or C9 polymeric surface in contact with water is plotted in Figure 1. When the temperature of the stationary phase, preconditioned with acetonitrile, was being raised, the change in polarity of the surface was linear with $1/T$. This behavior was followed, at a higher temperature, by a sigmoidal transition of the surface polarity towards less polar values. At still higher temperatures, the surface polarity again varied linearly with $1/T$. Upon cooling, the surface polarity increases with a linear dependence for temperatures as small as the initial conditions. Since the nonlinear portion of the curve is not repeated, the stationary phase at the end of the experiment is less polar than at the beginning. The temperature where the surface polarity undergoes a nonlinear change is dependent on the chain length of the surface-bound hydrocarbons. The temperatures characteristic of the on-set of this nonlinear region are 41 °C and 49 °C ($\pm 10^\circ\text{C}$) for the C8 and C9 polymeric surfaces, respectively.

Gilpin and coworkers [7,8,17] have noted a similar temperature dependence in the retention of phenol and resorcinol on C8, C9, and C10 stationary phases. Following preconditioning with pure organic modifier and elution with a totally aqueous mobile phase, increasing temperature causes the log of the capacity factor, $\ln k'$, to decrease linearly with $1/T$; at a chain length-dependent, transition temperature, $\ln k'$ undergoes a sigmoidal drop in magnitude. Following this transition, $\ln k'$ again varies linearly with $1/T$, along a line which is offset from the original temperature dependence. Two examples of the temperature dependent retention behavior of phenol and resorcinol from

Reference 7 have been reproduced in Figure 2, which allow the similarity between the retention data and the surface polarity to be compared (see Figure 1). Further verification of the strong correlation between these two experiments is the agreement of the transition temperatures which were observed; the on-set temperatures for the non-linear decrease in $\ln k'$ were reported as 40.7 °C and 51.8 °C ($\pm 1.5^\circ\text{C}$) for the C8 and C9 stationary phases, respectively [7]. The transition temperatures for surface polarity and solute retention, therefore, agree within their uncertainty bounds.

Surface structural changes with temperature. The temperature-dependent hysteresis in surface polarity and solute retention appears to be related to the preconditioning with organic modifier, in this case acetonitrile. By collecting and analyzing fractions of the aqueous mobile phase following the transition temperature, Gilpin and coworkers have elegantly showed that the conditioning solvent was initially present in the alkyl layer and released upon heating [8]. In interpreting this observation, however, the authors suggested that the solvent release was associated with a significant rearrangement of the alkyl chain conformation. They assert that the chains evolve from a "collapsed state" with "solvent entrapment within the bonded hydrocarbon layer during formation" to a more stable, chain-extended or "bristle" configuration where "entrapped conditioning solvent is released and replaced by water" [8,17].

The surface polarity change indicated by sorbed pyrene fluorescence is consistent with the observed loss of conditioning solvent from the interface, but not with a chain conformation where alkyl ligands extend into the aqueous solution. Although less polar than water, the preconditioning solvent is considerably more polar than the bound hydrocarbonaceous ligands. Removal of solvent molecules from the bound layer would decrease the average polarity of

the stationary phase as observed in the pyrene fluorescence. The irreversible decrease in polarity which takes place in the transition region can, therefore, be attributed to the departure of intercalated solvent from the stationary phase leaving a less polar environment. This interpretation is consistent with other spectroscopic studies of the effects of conditioning solvent on stationary phase environments [13,15,16]. On the other hand, the observed decrease in polarity upon heating confirms that the conditioning solvent is not replaced by water, at least in regions of the stationary phase occupied by a sorbed, hydrophobic solute. These results cast doubt about the existence of a extended chain or "bristle" configuration for the stationary phase layer under aqueous mobile phase conditions. Instead, the surface environment both before and after the transition temperature appears to exist in a collapsed state [4], where environment differences are primarily determined by the presence or absence of the preconditioning solvent. This interpretation appears to be reasonable in terms of hydrophobic interactions between the hydrocarbon ligands and water, and the capability of amphiphilic molecules such as typical reversed-phase organic modifiers to reduce surface tension at a hydrophobic layer-water and hydrophobic layer-silica interfaces. The results are supported by the theoretical predictions of a lattice model developed by Martire and Boehm [21].

In addition to the non-linear decrease in surface polarity accompanying the loss of conditioning solvent, a systematic lowering of the stationary phase polarity is observed as the system temperature is increased. This trend is observed both before and after the loss of conditioning solvent at the interface. To better understand this trend, the effect of temperature on the vibronic band ratios of pyrene fluorescence was measured in four solvents of

differing polarity, and the results are plotted in Figure 3. For each of the solvents, a similar reduction in the environmental polarity of the probe is observed with increasing temperature. This trend would indicate a lowering of solvent-induced perturbations which are responsible for mixing the weak L_b transition, which dominates the highest energy vibronic band, with the much stronger L_a transition in pyrene [18,19]. The slopes of the curves in Figure 3 are nearly identical suggesting a common mechanism underlying the response in all four solvents. The observed trends in polarity should correspond to a decrease in the density of the solvents with increasing temperature. This reduction in density would effectively increase the size of the solvent cage or the free volume of the solvent and thereby increase the average distance between pyrene and its perturbing neighbors, the final result being a decrease in the dielectric constant of the probe's immediate surroundings.

The density dependence of the vibronic band ratios for pyrene fluorescence has been included in an empirical response model by Stahlberg and Almgren [14] where the III/I intensity ratio of pyrene in a solvent was found to correlate well with the solvent's dipole moment divided by its molecular volume. The slope of the III/I ratio versus $1/T$ plot at a given temperature should therefore reflect the thermal expansion coefficient of the solvent. Plotting the solvent density on the same $1/T$ axis yields very similar curves with the same downward curving, inverse temperature dependence as seen in Figure 3 [22].

A similar decrease in the effective dielectric constant of pyrene's surroundings with increasing temperature is also observed in both sets of stationary phase data. The slopes of the surface environment curves in Figure 1 are of the same magnitude as the slopes of the free solution curves in Figure 3. While the stationary phase layer would not duplicate the behavior of

a free solution, the similarities in response are great enough to suggest an analogy. The environment of pyrene sorbed to a polymeric stationary phase responds to a change in temperature in a manner analogous to thermal expansion of a bulk solvent. Studies of the temperature dependence of monomeric stationary phases [23] reveal quite different behavior for these surfaces, where little sensitivity to temperature change is observed following loss of conditioning solvent. The lack of a response to temperature for the monomeric stationary phase is consistent with a symmetry perturbation of pyrene's excited states which is interfacial in nature and thus depends less significantly on the density of its surroundings. If differences in this behavior are similarly interpreted, then the slope of the C9 polymer stationary phase temperature dependence being steeper than C8 in Figure 1 could relate to how much of the probe's environment is of bulk versus interfacial character. Differences in the temperature dependence of surface environments, therefore, appear to carry information about surface structures and sorption mechanisms. More complete discussion of these issues follows in a separate manuscript [23].

Organic Modifier in Alkylated Silica Environments. The lowering of the polarity of C8 and C9 stationary phases upon loss of conditioning solvent provides strong evidence that the surface structure does not evolve from a collapsed to an extended configuration upon heating. As a result, a mechanism other than physical entrapment for the retention and loss of the conditioning solvent must be considered. Regardless of changes in alkyl chain conformation, physical entrapment of organic solvent by an alkyl layer is not a likely mechanism for solvent retention because of molecular diffusion. Taking the viscosity of the stationary phase from excimer formation rates to be about 19 cP [24], and the thickness of the hydrocarbon layer to be less than 2.0 nm,

diffusion rates through the alkyl layer are extremely fast. Even if the solubility of organic solvent in the hydrocarbonaceous overlayer were only 0.01% of its concentration at the underlying silica surface, the diffusion rate calculated from this viscosity and solubility reveals that >99% of "trapped" organic solvent would diffuse from the stationary phase into the mobile phase in less than 1 msec. Even if transport of the solvent through the stationary phase were as slow as molecular diffusion through a crystalline hydrocarbon solid [25], trapped solvent would still be released in less than one second. Physical entrapment, therefore, probably cannot account for solvent retention in the stationary phase, independent of changes in ligand conformation.

Another mechanism which could account for the retention of an amphiphilic solvent under these conditions relates to the reduction in surface tension of the alkyl chain-water interface and possibly the alkyl chain-surface silanol interface. The addition of mutually miscible solvent molecules at these interfaces lowers the surface free energy, and interfacial excess concentrations are, therefore, selectively adsorbed [26]. If this process were a simple two-phase adsorption equilibrium, however, the lack of organic modifier in the aqueous mobile phase would cause its slow but eventual loss from the stationary phase for any finite values of the adsorption equilibrium constant. An abrupt decrease in the concentration of retained solvent upon increasing the temperature could only arise if the enthalpy of adsorption were unrealistically large in magnitude.

These two observations, the stable retention of organic solvent and the well-defined temperature at which the solvent is lost from the surface phase, strongly suggest ordering of the solvated interface. While the irregular, porous structure of the underlying silica substrate would prevent long-range

order of bound ligands, nevertheless, short-range ordering of alkyl chains and intercalated solvent could support a stable, nematic phase which minimizes the surface energy. An analogy could be drawn between the short-range order of these bonded chains and the local intramolecular ordering of polymer molecules containing relatively long, linear side-chains; in free solution, these macromolecules have no long-range orientational order as in the porous silica matrix, but there exists strong correlation in the conformations of neighboring side-chains [26]. Above a particular temperature, one would expect such a system to exhibit a nematic-isotropic phase transition where conformational order is lost together with, in this case, the intercalated solvent.

Considerable supporting evidence exists in polymeric reversed-phase chromatographic materials for the formation of surface structures which have local conformational order. In Gilpin and Squires' pioneering observations of temperature dependent retention hysteresis in C8, C9, and C10 phases [7], it was found that observation of the hysteresis required a significant surface coverage by alkyl chains, and that the magnitude of the hysteresis increased dramatically with increasing % carbon above a critical coverage. Studies of the effects of temperature on densely-grafted C22 [28] and C18 [29] polymer phases showed similar, persistent effects of conditioning solvent on elution with aqueous mobile phase and temperature-dependent hysteresis in retention.

Wise and Sander [11,12,30] have showed that C18, polymer stationary phases grafted at high-carbon loadings on large-pore silica supports produce unusually large selectivity for planar solutes compared to monomeric phases; drawing on an analogy to retention on liquid crystalline phases in gas chromatography, they conclude that such polymeric phases are considerably more "ordered". The relationship between the observed selectivity and the pore diameter of the

substrate argues for conformational order over a significant range. Such ordering also requires a high density of alkyl ligands with a surface configuration produced by polymeric bonding of the phase. Similar conclusions can be drawn from a comparison of the temperature-dependent retention and sorption environment hysteresis in C6, C7, and C8 monomeric stationary phases, where neither the persistent retention of conditioning solvent nor a distinct transition temperature for its accelerated loss are observed [23].

While the pyrene vibronic ratios show an effect of sorbed acetonitrile on the polarity of the stationary phase environment, it is clear from differences in solute retention [7] as shown in Figure 2 that the stationary phase affinity for other solutes is also affected. Since the mobile phase is the same for both upper and lower curves of Figure 2, differences in retention reflect only changes in the partial molar free energy of a solute in the stationary phase after correcting for changes in the phase volume ratio [27,28]. From the effects of the acetonitrile on the surface polarity, one might logically expect that the enthalpy of sorption of molecules such as phenol and resorcinol would be increased by the presence of acetonitrile the hydrocarbon layer. The slopes of the straight-line portions of the respective upper and lower curves of Figure 2, however, do not differ from each other by more than their error of estimation when fit to a linear equation [31]. The partial molar enthalpy of sorption of these molecules, proportional to the slope of the $\ln k'$ versus $1/T$ plot, does not change significantly upon loss of the conditioning solvent. This result could arise from the sorbed organic solvent residing primarily at the hydrocarbon-water interface, since calculations based on a lattice model have revealed [21] that adsorbed solvent minimally affects the solute distribution process compared to solvent in the interior of the stationary phase.

Isotherms for polar organic solvents adsorbed to the hydrocarbon interface predict that the adsorbed fraction remains high until the concentration of the organic solvent drops to nearly zero in an aqueous mobile phase [21]. The role of such solvent molecules in minimizing the surface tension at the aqueous interface has been discussed above.

In contrast to the insignificant differences in slope, the intercepts of the straight-line portions of respective upper and lower curves of Figure 2 are statistically distinguishable. The differences are equivalent to a lowering of the partial molar entropy of sorption by about $-2.0 (\pm 1.0)$ cal/mole $^{\circ}\text{K}$, before correcting for changes in the phase volume ratio. Using quantitative data for the amount of lost solvent [8], the carbon loading of the stationary phase and an estimate of its mass from the column size and packing method [7], the change in volume of the stationary phase due to loss of organic solvent would be less than 5% (corresponding to a 2% gain in mobile phase volume). The resulting change in phase ratio would account for less than 10% (-0.2 cal/mole $^{\circ}\text{K}$) of the change in partial molar entropy of sorption upon loss of solvent. The lowering of the sorption entropy upon loss of the preconditioning solvent supports the existence of a more ordered stationary phase in the preconditioned state, in the presence of the adsorbed organic solvent. Under these conditions, the effect of sorption of a solute molecule into the stationary phase layer is to locally disrupt this order and to increase the overall entropy of the system. Above the transition temperature, when the ordered stationary structure is lost, the effect of solute sorption on the stationary phase order is less and the partial molar entropy of sorption is lowered. Thus, thermodynamic data available from temperature-dependent retention of solutes support the idea of a structured, stationary phase layer in the presence of a conditioning solvent.

The overall (thermodynamic) stability of an ordered, solvated interface for polymeric stationary phases below a specific transition temperature would determine whether the temperature-dependent transition from this condition is reversible. If the solvated structure of the interface does not reversibly appear upon lowering the temperature, then the preconditioned surface is not likely a lowest energy configuration for the interface, but is a product of the rapid change in solvent conditions and only "kinetically" stable like a glass or supercooled liquid. The apparent irreversibility of the transition seen in Figures 1 and 2 is, however, not a true reflection of the thermodynamics of the surface structure since the released organic solvent was constantly continually flushed from the sample with 100% water during the experiment. Since the organic solvent component of the solvated interface is removed from the mobile phase, a reverse transition to the solvated interface cannot take place.

In order to observe whether a solvated, structured interface can reform as one lowers the temperature, the organic solvent must be present in the mobile phase, perhaps above a minimum concentration which would depend on the entropy change of the transition. Some evidence for the "reversibility" of the temperature-dependent transition exists from this work and from the literature. In order to control the amount of pyrene probe on the alkylated surface in this work, an equilibration step was added where pyrene in a 50:50 acetonitrile-water solution was exposed to the surface. Thus, the minimum solution concentration for maintaining equilibrium with a solvated surface structure is less than 50% acetonitrile. Furthermore, the change in solvent composition to 100% water takes about two minutes, based on observations of the refractive index gradients in the packed cell. Since interfacial diffusion is quite efficient on this time-scale as predicted above, the reservoir of acetonitrile

in the mobile phase required to equilibrate or to reform an order, solvated interface could be quite small. A small solution concentration of acetonitrile required to reach equilibrium with a structure interface may be reasonable in light of its anomalous tendency, at relatively low solution concentrations, to expel water from a polymeric stationary phase [32]. This tendency correlates well with the change in stationary phase polarity observed in this work. The apparent ability of this and other solvents to adsorb to the densely-grafted alkyl ligands at an aqueous interface allows an ordered, solvated structure to form. Experiments to more critically test the reversibility of this process are planned.

ACKNOWLEDGMENTS

This work was supported in part by the Office of Naval Research. Fellowship funds (to J.M.H.) from the Alfred P. Sloan Foundation are also acknowledged.

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FIGURE CAPTIONS

1. Temperature-dependent stationary phase polarity as measured by the fluorescence vibronic band ratios of pyrene sorbed to a) C8 and b) C9 polymeric surfaces, in contact with 100% aqueous mobile phase. Upper curve represents surface polarity immediately after preconditioning with acetonitrile. Lower curve represents surface polarity following temperature excursions above 41 °C, for C8, and 49 °C, for C9, respectively.
2. Temperature-dependent retention of a) phenol and b) resorcinol on C10 polymeric reversed phases with 100% aqueous mobile phase; surfaces were preconditioned with acetonitrile. Data are replotted from Figures 1c and 2c of reference 7.
3. Fluorescence vibronic band ratios of pyrene as a function of temperature in four solvents: a) water, b) acetonitrile, c) 1,2 ethanediol, d) decane. Precision of the data in curves a) - c) is about the size of the plotted points; reproducibility of the data in curve d) is indicated by the error bars.

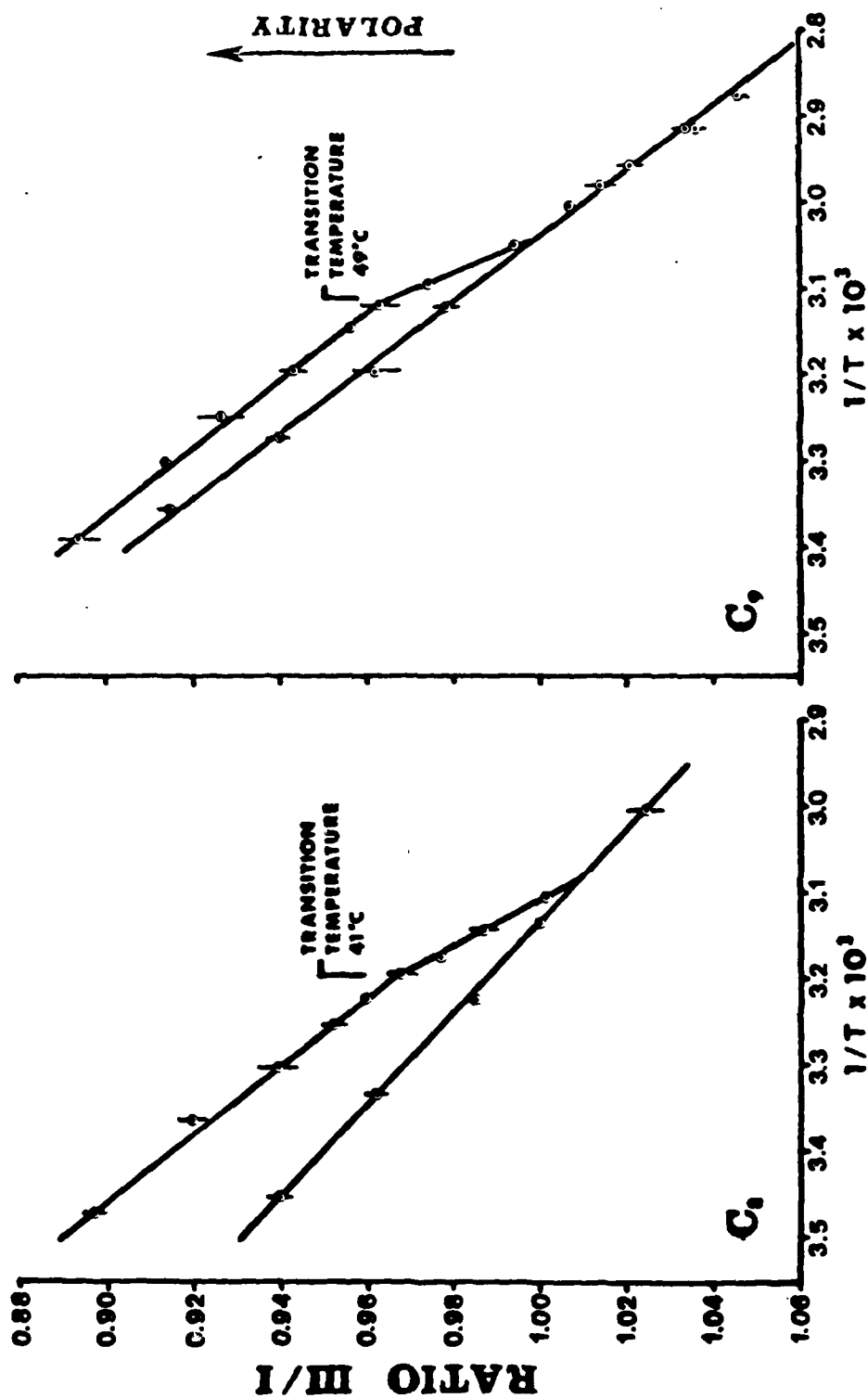


Figure 1

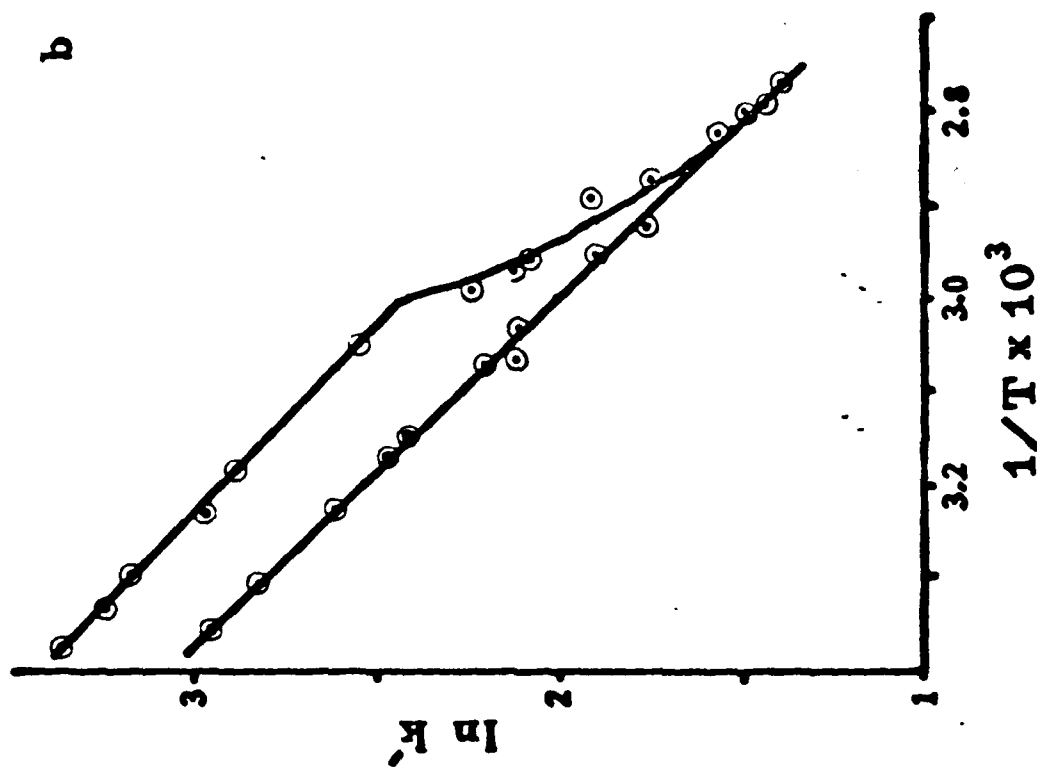
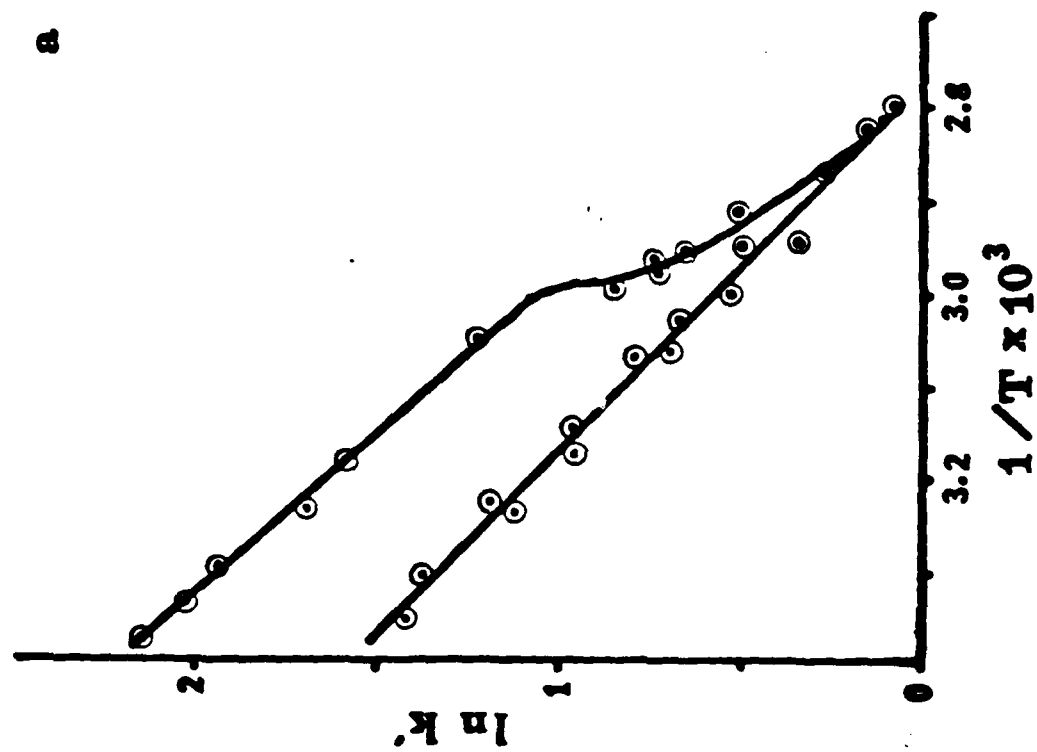


Figure 2

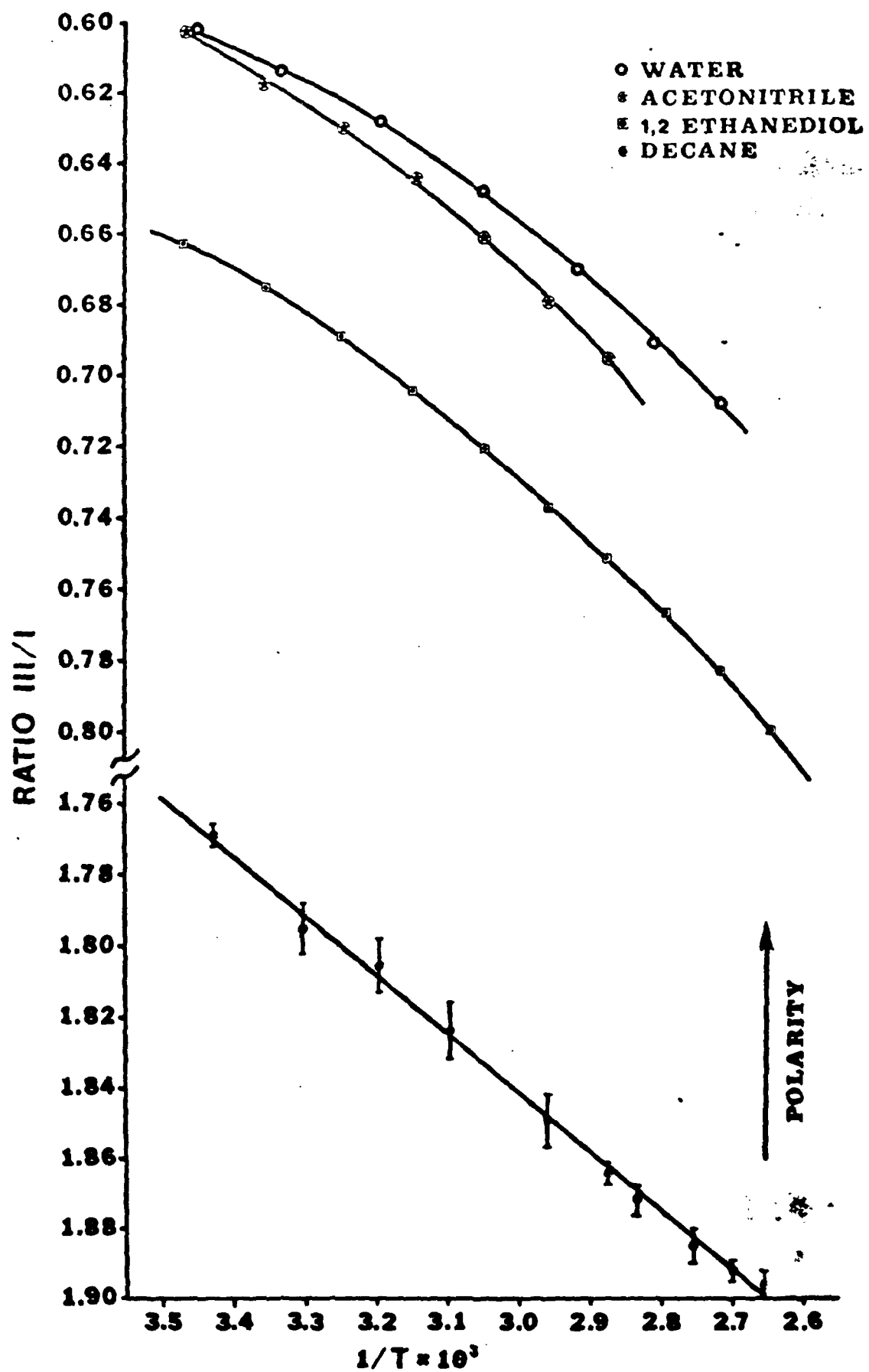


Figure 3

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